

## The subspecific status of *Pieris napi* (Pieridae) within the British Isles

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**Summary.** Previously, *Pieris napi* (Linnaeus, 1758) within the British Isles has been divided into different subspecies and also separated from mainland European populations on the basis of androconial and wing morphology variation. Using image analysis we obtained quantitative data on androconial scale shape measurements and wing morphology characters (size and colour pattern elements) of *P. napi* from the British Isles and France (wing morphology only) to examine the subspecific status of *P. napi* within the British Isles. Androconia are variable in shape but this variation is normally distributed. There is no basis for describing different scale types within the British Isles. Variation within populations in Scotland and southern England is greater than between regions and there is no basis for using androconial measures to describe Scottish specimens as subspecies. Wing size, shape and colouration are variable within populations and variation in particular characters is not consistent between generations or geographic regions. Wing morphology is a poor taxonomic tool for describing regional forms. We conclude that there is no evidence to divide *P. napi* in the British Isles into subspecies or to differentiate populations in the British Isles from mainland Europe.

**Key words.** Lepidoptera, Pieridae, taxonomy, biogeography, British Isles, androconia, morphology, image analysis, *Pieris napi*.

### Introduction

Morphological variation within species can be a response to current selection processes and/or the result of historic patterns of range changes and past patterns of isolation and divergence. Assessments of the effects of selective processes in different locations and biogeographic inference require reliable and quantitative estimates of trait variation at the morphological and/or genetic levels.

The *Pieris napi* (Linnaeus, 1758) complex has a number of different geographic forms within its Holarctic distribution (Geiger & Shapiro 1992). Within the Palaearctic, *P. napi* and closely related species are widespread. *P. napi* is seasonally and geographically variable and there is considerable confusion and uncertainty about the taxonomic status of most geographic forms, including specific and subspecific divisions. Within the British Isles the nominate species, *P. napi napi*, is described as absent (Emmet 1989) and three subspecies have been named: *sabellicae* (Stephens, 1827), type locality England, which is described as occurring within southern and northern Britain; *britannica* (Müller & Kautz, 1939), type locality Ireland, in Ireland and Scotland, and *thomsoni* (Warren, 1968), type locality Dunblane, in Scotland. Originally the basis for the separation of *sabellicae* and *britannica* from each other and from the nominate species was on differences of wing shape, colour and pattern expression and, in the case of *thomsoni*, on androconial variation. Warren (1961, 1968) originally described Scottish *P. napi* as having four androconial scale types but Thomson (1970, 1980) identified two further scale types in Scottish populations and only one in specimens from southern England. The occurrence of different scale types and a comparison with

androconia from other geographic regions led Warren (1968) and Thompson (1980) to conclude that Scottish populations were more similar to *P. napi adalwinda* (Fruhstorfer, 1909), type locality Finnmark, with a distribution north of 65°N in Fennoscandia. Subsequently Bowden (1983) identified Irish specimens as having androconial scale types similar to those of *thomsoni*.

Described morphological and androconial variation has been used as supporting evidence for a double invasion of *Pieris napi* into the British Isles during the Holocene (Dennis 1977). According to him, early arriving (15,000–13,000 years BP), cold tolerant *P. napi* survived the Younger Dryas and spread northward with warming at the beginning of the Holocene (11,500 years BP to present), but were replaced by *P. napi* from more southerly locations in southern Britain. It was also suggested that if two forms exist there has been interbreeding, providing a mosaic of populations in Scotland (Dennis 1977; Bowden 1983).

Studies of allozymes from *P. napi* in Scotland and northern England reveal non-equilibrium in respect to gene flow and genetic drift (Porter & Geiger, 1995). This has been interpreted as the result of secondary contact between a northern population group and more recently invading populations, consistent with the hypothesis of Dennis (1977), or genetic isolation within population sets within northern parts of the British Isles.

The morphological variation which has been used to elevate regional populations to subspecific status has been on the basis of comparing relatively few individuals from a species which is known to display within-population variation, some of which is related to the rate of pupal development (Thompson 1947) and thus temperature. There has also, in the case of *britannica*, been an emphasis on the yellow ground colour revealed by breeding experiments to be the product of more than one recessive allele also present in other parts of the British Isles (Emmet 1989). Described androconial variation has either been qualitative or when quantitative, based on visual examination (Bowden 1983) and not subject to statistical analysis. Using limited qualitative data is poor taxonomic practice to employ in such a variable species. Here, we use a quantitative analysis of morphological and androconial variation of *P. napi* to provide a reassessment of its status in the British Isles.

## Methods

**Androconial measurements.** Neither Warren (1968), Thomson (1970, 1980) or Bowden (1983) provided any information about the wing area, which they removed androconia from (they just stated that these were taken from the upper surface), or used any quantifiable criteria to define 'scale types'. Assessment of scale types was based on visual inspection, but from published illustrations the main difference between types is on the basis of shape, principally scale length and width, especially midway between the base and apex. Bowden (1983) also stated that some scale types could occur at low frequency (2–3 %) within any individual. We use a quantitative approach using individuals from eight Scottish and six southern British sampling locations (Table 1). Preliminary observations confirmed the presence of androconia over the whole of the fore and hind wing upper surfaces, with more on the forewing.

**Tab. 1.** Sampling locations and sample sizes of individuals of *Pieris napi* used for androconial and wing morphology measurements

	Location	Coordinates <sup>1</sup>	Sampling date	Androconial sample (N)	Morphology sample	
					Males	Females
<b>Scotland</b>						
Spring	Loch Aline	NM702473	05/1998	10		
	Breacleit	NB155376	05/2001		20	20
Summer	Loch Aline	NM702473	07/1996	1		
	Glen Lonan	NM938280	07/1996	2		
	Glen Achulish	NN045583	07/1996	2		
	Barcaldine	NM962411	07/1996	2		
	Sneils	NM998577	07/1996	1		
	Glas Drum	NN009461	07/1996	1		
	Ford	NN023788	07/1996	1		
	Breacleit	NB155376	07/1996	1		
				07/2000		20
<b>Southern England</b>						
Spring	Long Crendon	SP6839093	05/2000		5	
	Shotover	SP566058	05/2000			1
	Lye Valley	SP549060	05/2000		15	13
Summer	Shotover	SP566058	08/2000 & 2001	4 2	13 10	11 10
	Lye Valley	SP549060	08/2000 & 2001	1 1		
	Loosley Row	SP816011	07/1996	2		
	Cothill Fen	SU465996	08/1999			
	Buckfastleigh	SX7366	08/1996			
<b>Southern France</b>						
Summer	St. Andeol	44°45'N 05°22'E	06/1999		12	10
	Gumaine	44°45'N 05°22'E	06/1999		2	1
	Menée	44°45'N 05°22'E	06/1999		9	1
	Bois de Tauligan	44°45'N 05°22'E	06/1999			11

<sup>1</sup> National Grid Reference for the British Isles; latitude and longitude in France

Using a fine paintbrush, scales were removed from the cell of the upper forewing and then gently tapped onto a microscope slide. This was done separately for 10 males from each sampling location. These were then examined using a Zeiss<sup>TM</sup> bifocal microscope at 200-fold magnification. Images of the first 100 androconia from each individual which were flat were then captured using a digital camera (JVC KY-F55B) attached to a frame grabber (Imaging Technology IC-PCI) and stored for subsequent analysis using OPTIMAS<sup>TM</sup> (v.6.0) imaging software. Measurements of length, mid-width and neck width were taken for each androconial scale. From these, three shape describing variables were calculated; length/mid-width ratio, length/neck-width ratio and mid-scale/neck-width ratio. In addition, forewing length from the wing base to apex was also measured using the same camera, framegrabber and software. All measurements were made in calibrated measurements and exported to Statistica version 5.5 (Statsoft 1999) for subsequent analysis. Repeated measures for all variables gave a

reliability of 95%. Populations and generations were compared using MANOVA (multivariate analysis of variance) using all androconial scale variables. The test statistic Wilk's  $\lambda$  (determinant of the within groups variance/covariance matrix over the determinant of the total variance/covariance matrix) was used to compare between and within region/generation variation. Wilk's  $\lambda$  scales from 0 (perfect discrimination) to 1 (no discrimination). Regional and seasonal variation is visualised using non-metric multidimensional scaling of an individual  $\times$  individual matrix of Spearman rank correlation coefficients produced from values of (neck-width  $\times$  mid-width)/length for all androconia measured from single individuals.

**Wing colour and pattern.** Digital colour images were captured of both surfaces of wings removed from adults from first and second generation adults from Scottish and southern English locations and second generation individuals from French locations (Table 1). Previous distinctions between different named subspecies have been on the basis of the intensity of yellow ground colour and vein colouration (black/grey/green) of the hindwing lower surface; the intensity and area of basal area melanisation of forewing and hindwing upper surfaces and wing sizes and shapes of both sexes (Stephens 1827; Verity 1916; Müller & Kautz 1939; Emmet 1989). Other distinctions have also been made for individual subspecies, such as the size and colour of postdiscal spots in female *sabellicae*. We took quantitative measurements of wing characteristics (Table 2) that have been described for all named subspecies within the British Isles. For image analysis, wings were illuminated with a Zeiss™ fibre optic ring light and captured and processed with the same camera and software used for androconial measures. All images were calibrated and repeat measures gave a reliability of at least 95%. Colour was measured in the red, green and blue planes. Each plane has a separate luminance value ranging from 0 (none) to 255 (complete saturation). (Pure black = 0:0:0 and pure white = 255:255:255). For the analysis of 'white/yellow' and black in this study, the threshold values of 200–235:200–225:157–195 for white/yellow and 0–177:0–184:0–129 were applied. Mean luminance values of wing components in each of the thresholded bands for each colour were used in subsequent analyses. Because we randomly sampled from wild populations not all captured specimens were perfect and intact. Thus measurements of fore- and hindwing dorsal and ventral surfaces within populations were not all from the same individuals, with approx. 5% being taken from different individuals. As field sampling was random and our analysis is primarily concerned with between population variation such an approach is justified. Wing morphology comparisons were made using ANOVA.

## Results

**Androconial variation.** There was no relationship between the mean values of the three basic androconial measurements of length, neck width and mid-length width of any individual with wing area for any geographic or seasonal sample or for all samples (Table 3). Thus, all further analysis is of unscaled measurements. Within any region or brood, there is no evidence for multimodality in any measure. All variables are normally distributed (Kolmogorov-Smirnov tests,  $P > 0.2$  in all cases) in-

**Tab. 2.** The wing morphology characteristics used to quantitatively compare *Pieris napi* from Scotland, England and southern France and their use by previous authors to distinguish subspecies.

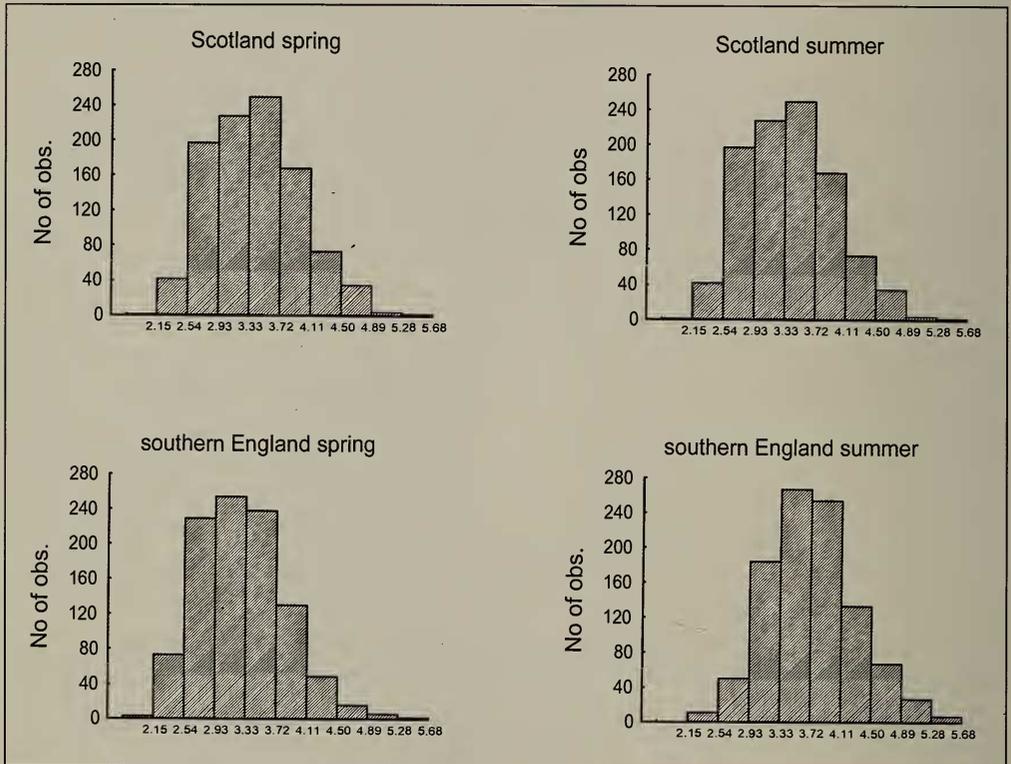
Wing surface	Wing character measured	Previous use in describing subspecies
Fore and hindwing	Wing area Area : perimeter length ratio	Used by Stephens (1827) to distinguish <i>sabellicae</i> from <i>napi</i> , the former described as having more angular wings
Hindwing ventral surface	Luminance of yellow colouration Proportion of wing yellow Melanisation of veins Extent of black scales over veins	Stephens (1827) and Verity (1916) describe <i>sabellicae</i> as yellower and more melanised than <i>napi</i> . Warren (1968) and Thomson (1970) describe a greater frequency of yellow forms in <i>thomsoni</i> than <i>sabellicae</i>
Forewing dorsal surface	Brightness of white background colour Melanisation of veins Extent of black scales over veins Melanisation of basal area	Stephens (1827) and Verity (1916) describe <i>sabellicae</i> as brighter than <i>napi</i> , with greater and more extensive melanisation; Müller & Kautz (1939) and Warren (1968) describe <i>thomsoni</i> as yellower and more melanised than <i>sabellicae</i> on forewing and hindwing dorsal surfaces
Hindwing dorsal surface	Brightness of white background colour Melanisation of veins Extent of black scales over veins Melanisation of basal area	

**Tab. 3.** Androconial length, neck and mid-scale widths and ratios of widths to length and mid-scale to neck width ratios of specimens of *Pieris napi* from Scotland and southern England. All means are reported  $\pm 1$  SD. N.S. – not significant ( $P > 0.05$ ).

G1 – first generation; G2 – second generation;  $r_s$  – Spearman’s rank correlation of individual mean measurement with forewing area;  $P$  – significance of  $r_s$

Androconial variable	Region and generation												Overall relationship with wing area	
	Scotland						Southern England							
	G1	$r_s$	$P$	G2	$r_s$	$P$	G1	$r_s$	$P$	G2	$r_s$	$P$	$r_s$	$P$
Length (mm)	0.092 $\pm 0.007$	0.14	N.S.	0.092 $\pm 0.007$	-0.32	N.S.	0.093 $\pm 0.007$	0.43	N.S.	0.092 $\pm 0.007$	-0.08	N.S.	0.29	N.S.
Mid-scale width (mm)	0.029 $\pm 0.004$	-0.28	N.S.	0.029 $\pm 0.004$	0.32	N.S.	0.029 $\pm 0.004$	0.09	N.S.	0.029 $\pm 0.004$	0.25	N.S.	-0.25	N.S.
Neck width (mm)	0.026 $\pm 0.004$	-0.28	N.S.	0.026 $\pm 0.003$	0.32	N.S.	0.026 $\pm 0.004$	0.05	N.S.	0.026 $\pm 0.004$	0.25	N.S.	-0.19	N.S.
Length/mid-scale width ratio	3.26 $\pm 0.55$			3.25 $\pm 0.54$			3.27 $\pm 0.56$			3.27 $\pm 0.55$				
Length/neck width ratio	3.61 $\pm 0.62$			3.60 $\pm 0.61$			3.63 $\pm 0.63$			3.62 $\pm 0.62$				
Mid-scale/neck width ratio	1.11 $\pm 0.13$			1.11 $\pm 0.13$			1.11 $\pm 0.13$			1.11 $\pm 0.13$				

cluding the ratio of androconial length to mid-length width (Figure 1) and the ratio of width measurements, both of which are the most likely measures to categorise androconial scale type classes. Overall, there are no significant differences between the distributions of androconial scale dimensions from the Scottish and southern English regions.



**Fig. 1.** Distributions of the ratios of androconial length to mid-width for spring and summer generations of *Pieris napi* from Scotland and southern England.

MANOVA (multivariate analysis of variance) is used to examine within and between region and season differences of androconial size and shape measures (Table 4). Within any region and season there are differences between individuals in androconial size and shape measures. For both Scotland and southern England there are greater differences between samples within a season than between seasons (Scotland, Wilk's  $\lambda$  0.77 versus 0.16 and 0.07; southern England Wilk's  $\lambda$  0.66 versus 0.10 and 0.12). In addition, differences between regions are no greater than within regions (Wilk's  $\lambda$  0.67 versus 0.77 and 0.66). This is indicative of between individual variation exceeding between season or region variation.

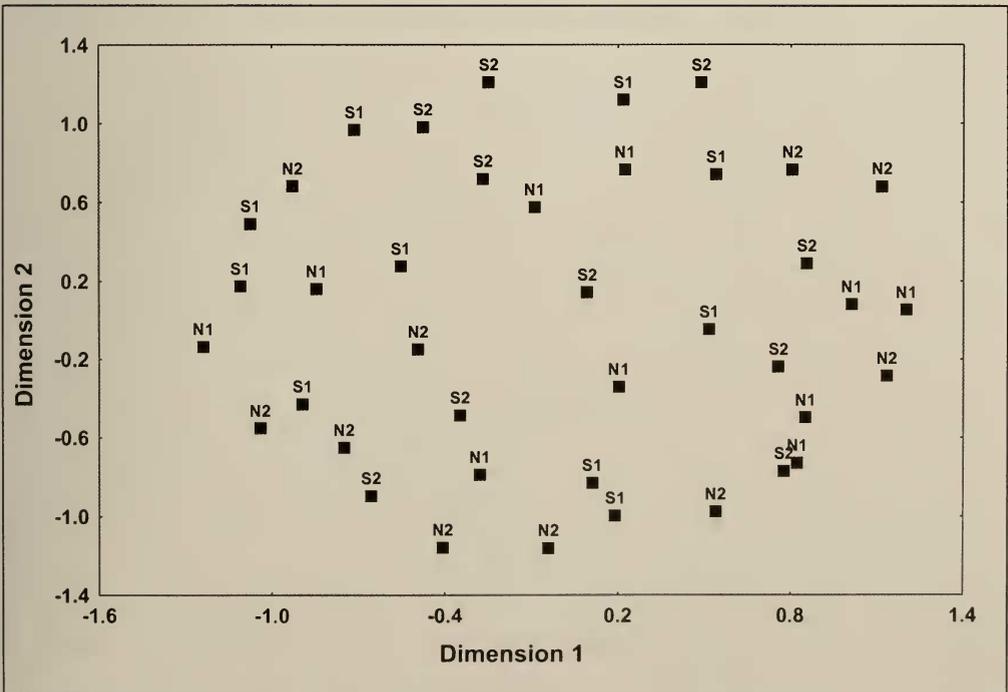
A two dimensional non-metric scaling plot derived from an individual  $\times$  individual matrix of Spearman correlation coefficients produced from individual values of androconial neck-width  $\times$  mid-width)/length reveals a lack of any underlying geo-

**Tab. 4.** MANOVA comparisons of within region and between region androconial variation, using all androconial scale variation measures. Sample sizes are 10 individuals from each region and 100 scales from each individual.

Comparison	Wilk's Lambda	Rao's R	P
Scotland spring	0.16	95.3	<0.01
Scotland summer	0.07	161.1	<0.01
southern England spring	0.10	129.1	<0.01
southern England summer	0.12	116.7	<0.01
Scotland spring vs summer	0.77	193.2	<0.01
England spring vs summer	0.66	339.0	<0.01
Scotland vs southern England	0.67	195.6	<0.01

Wilk's Lambda is the determinant of the within groups variance/covariance matrix over the determinant of the total variance/covariance matrix. It ranges from 0, perfect discrimination, to 1, no discrimination. Rao's R is a transformed value of Wilk's Lambda to determine the significance of each effect. It follows the *F*-distribution. *P* - Significance of Rao's R.

graphic or between-population structuring in androconial scale variation (Figure 2). No grouping of specimens on the basis of location is evident, dimensional distances between individuals from the same location are as large as those between individuals from different locations.



**Fig. 2.** Two-dimensional non-metric scaling plot of Scottish (N) and southern English (S) androconial scales from first (1) and second (2) generations derived from an individual × individual Spearman correlation matrix derived from androconial length and width measurements.

**Tab. 5.** Mean wing morphology measures of *Pieris napi* from Scotland, southern England and southern France and statistical comparisons between generations within regions and between regions and generations.

Variable		Scotland		southern England		southern France	F & P (between region and generation)
		G1	G2	G1	G2	G2	
FW area (mm <sup>2</sup> )	male	209.2 ± 20.1	215.0 ± 22.0	182.6 ± 21.5	218.5 ± 23.0	252.7 ± 24.1	F <sub>(4,95)</sub> = 35.5 P < 0.001
	female	203.6 ± 20.6	195.3 ± 9.4	176.0 ± 21.2	193.5 ± 17.3	242.9 ± 14.0	F <sub>(4,88)</sub> = 19.2 P < 0.001
FW area: perimeter length ratio	male	3.5 ± 0.2	3.6 ± 0.1	3.3 ± 0.2	3.6 ± 0.2	3.9 ± 0.2	F <sub>(4,95)</sub> = 32.8 P < 0.001
	female	3.5 ± 0.2	3.5 ± 0.1	3.2 ± 0.2	3.4 ± 0.2	3.9 ± 0.1	F <sub>(4,88)</sub> = 42.4 P < 0.001
HW area (mm <sup>2</sup> )	male	226.3 ± 20.5	230.8 ± 30.9	205.3 ± 19.8	233.4 ± 15.4	250.8 ± 24.5	F <sub>(4,94)</sub> = 23.7 P < 0.001
	female	213.7 ± 22.4	212.6 ± 17.5	190.9 ± 26.0	208.4 ± 21.1	262.5 ± 33.6	F <sub>(4,88)</sub> = 22.9 P < 0.001
HW area: perimeter length ratio	male	3.9 ± 0.2	4.1 ± 0.1	3.8 ± 0.2	4.0 ± 0.3	4.3 ± 0.3	F <sub>(4,94)</sub> = 17.7 P < 0.001
	female	3.8 ± 0.2	3.8 ± 0.1	3.6 ± 0.3	3.8 ± 0.2	4.2 ± 0.3	F <sub>(4,88)</sub> = 20.3 P < 0.001
HW ventral yellow luminance	male	177.0 6.5	179.2 ± 2.7	180.5 2.3	184.0 ± 0.6	182.8 ± 2.6	F <sub>(4,95)</sub> = 11.3 P < 0.001
	female	174.0 4.1	175.4 ± 1.2	180.6 3.4	180.7 ± 2.8	180.7 ± 2.9	F <sub>(4,87)</sub> = 23.3 P < 0.001
HW ventral surface proportion yellow	male	0.22 ± 0.02	0.26 ± 0.01	0.27 ± 0.02	0.33 ± 0.02	0.41 ± 0.04	F <sub>(4,95)</sub> = 10.7 P < 0.001
	female	0.16 ± 0.03	0.13 ± 0.03	0.33 ± 0.03	0.54 ± 0.03	0.63 ± 0.02	F <sub>(4,87)</sub> = 68.4 P < 0.001
HW ventral surface vein melanisation (luminance)	male	125.3 ± 3.8	127.3 ± 2.6	133.2 ± 2.7	130.6 ± 3.5	127.3 ± 4.3	F <sub>(4,95)</sub> = 16.6 P < 0.001
	female	122.4 ± 3.6	122.5 ± 3.1	129.1 ± 2.9	126.8 ± 3.2	127.6 ± 4.3	F <sub>(4,87)</sub> = 6.6 P < 0.001
HW ventral proportion of wing covered by black scales	male	0.59 ± 0.05	0.52 ± 0.07	0.51 ± 0.04	0.36 ± 0.13	0.13 ± 0.08	F <sub>(4,95)</sub> = 106.9 P < 0.001
	female	0.53 ± 0.09	0.46 ± 0.10	0.49 ± 0.06	0.23 ± 0.81	0.07 ± 0.03	F <sub>(4,87)</sub> = 112.3 P < 0.001
FW dorsal white luminance	male	200.4 ± 7.7	199.0 ± 0.5	199.0 ± 0.5	198.9 ± 4.4	199.0 ± 0.5	F <sub>(4,95)</sub> = 1.73 P > 0.05
	female	176.5 ± 60.3	179.9 ± 61.5	200.2 ± 0.8	199.5 ± 0.7	200.2 ± 0.5	F <sub>(4,88)</sub> = 1.7 P > 0.05
FW dorsal vein melanisation (luminance)	male	125.9 ± 6.1	136.1 ± 3.4	135.9 ± 4.7	137.7 ± 3.3	141.8 ± 3.8	F <sub>(4,95)</sub> = 37.8 P < 0.001
	female	113.1 ± 19.5	134.4 ± 3.8	131.9 ± 4.8	134.8 ± 6.0	138.8 4.7	F <sub>(4,88)</sub> = 15.5 P < 0.001
FW dorsal proportion of wing covered by black scales	male	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.11 ± 0.01	F <sub>(4,95)</sub> = 1.1 P > 0.05
	female	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	F <sub>(4,88)</sub> = 14.7 P < 0.001
FW basal area melanisation (luminance)	male	125.2 ± 5.0	136.3 ± 3.4	135.7 ± 4.4	138.0 ± 3.3	141.7 ± 3.6	F <sub>(4,95)</sub> = 48.0 P < 0.001
	female	113.2 ± 19.0	133.2 ± 4.5	131.9 ± 4.8	135.0 ± 5.9	139.0 ± 4.5	F <sub>(4,88)</sub> = 20.6 P < 0.001

## Continued Tab. 5.

HW dorsal white luminance	male	195.1 ± 3.7	197.3 ± 0.8	197.1 ± 0.4	197.6 ± 1.0	198.6 ± 0.6	F <sub>(4,95)</sub> = 8.94 P < 0.001
	female	195.8 ± 3.3	198.6 ± 1.7	199.2 ± 0.6	197.6 ± 0.8	200.0 ± 0.9	F <sub>(4,88)</sub> = 12.1 P < 0.001
HW dorsal vein melanisation (luminance)	male	134.0 ± 6.1	141.1 ± 3.2	139.3 ± 3.1	141.4 ± 2.3	141.2 ± 3.0	F <sub>(4,95)</sub> = 14.25 P < 0.001
	female	139.8 ± 3.9	140.5 ± 2.6	140.1 ± 4.6	141.6 ± 2.5	138.8 ± 3.2	F <sub>(4,88)</sub> = 2.02 P > 0.05
HW dorsal proportion of wing covered by black scales	male	0.19 ± 0.08	0.17 ± 0.03	0.15 ± 0.02	0.11 ± 0.03	0.08 ± 0.03	F <sub>(4,95)</sub> = 18.75 P < 0.001
	female	0.39 ± 0.18	0.32 ± 0.08	0.31 ± 0.12	0.15 ± 0.04	0.07 ± 0.03	F <sub>(4,88)</sub> = 32.5 P < 0.001
HW dorsal basal area melanisation (luminance)	male	125.2 ± 5.7	137.3 ± 3.4	135.1 ± 3.9	145.1 ± 17.3	140.2 ± 3.3	F <sub>(4,94)</sub> = 14.6 P < 0.001
	female	130.4 ± 4.8	137.9 ± 3.1	136.7 ± 3.46	139.7 ± 3.5	137.1 ± 3.8	F <sub>(4,88)</sub> = 17.5 P < 0.001

Wing morphology variables are normally distributed and their variation is summarised in Table 5. (All comparisons are supported by LSD post-hoc tests,  $P < 0.05$ ). Within any region there is some seasonal variation in characteristics but seasonal variation is not consistent between regions, or between sexes within regions. Males are smaller (forewing and hindwing) in the first generation compared to the second in Scotland and in southern England. For females, seasonal size variation is more complex. In Scotland they are smaller in the second generation, but in southern England they are larger in the second generation. Second generation individuals also have more rounded wings (area/perimeter ratios) than those of the first. First generation individuals of both sexes have darker melanised basal wing areas on the upper wing surfaces than second generation individuals in Scotland, but not in southern England where this difference is restricted to females only. Upper wing surfaces are brighter in the first than second generation in both Scotland and England. Upper surface melanisation, excluding basal melanisation, is greater in the first generation in Scotland and only on the hindwing for males in the first generation in southern England. The extent of dark scales on the dorsal surfaces is greater in the first generation in both Scotland and southern England for both sexes. Some individuals had yellow suffusion on the ventral hindwing. For both sexes in Scotland and for females in southern England there were no seasonal differences in either the intensity of yellow or its extent. Males from southern England had more extensive and darker yellow underside colouration in the second generation than the first. In both regions and sexes the veins on the hindwing underside were more heavily melanised in the spring than the summer in both intensity and extent.

Comparisons including the French samples are more complex. Within males, first generation individuals differ between regions in size, but second generation males do

not differ in size between Scotland and southern England but are larger in southern France. Females of the first generation are larger in Scotland than southern England. In the second generation they are the largest in France and the smallest in southern England. Females of the second generation from southern England do not differ in either forewing or hindwing shape from females from France but those from the first generation do. There are also no significant differences in shape between females from Scotland and from southern England for either generation. In contrast, males differ in shape between the regions in spring, and in summer between Scotland and southern England but not between southern England and France. Dorsal basal wing area melanisation is darker in first generation males and females in southern England than in Scotland. This characteristic does not differ between Scotland, southern England and southern France in the second generation. The ventral ground colour of both males and females is brighter yellow in the first generation in Scotland than in southern England but there are no significant differences in this characteristic in males between the three regions in the second generation. The ventral hindwing of second generation females is brighter yellow in Scotland than elsewhere but does not differ between southern England and southern France. In both generations the darkness of the wing venation of the ventral hindwing of both sexes does not differ between regions, but the dark scales surrounding the veins are the most extensive in Scotland and the least extensive in southern France. In the first generation in Scotland the yellow ground colour of both sexes and female dorsal basal melanisation is more variable than in any other region or generation.

Regional and seasonal comparisons reveal an overall pattern of variability and a lack of consistency in the way individual characteristics vary.

## Discussion

Our quantitative findings on androconial and wing morphology variation within the British Isles are not consistent with earlier work (e.g., Stephens 1827; Verity 1916; Müller & Kautz 1939; Warren 1961, 1986; Thomson 1970, 1980). The only consistent finding between our measures of androconia and previous ones is the lack of a relationship between forewing size and androconial scale size (Warren 1961). All the measures of androconial shape we have made are normally distributed and variation within Scottish samples is no greater than in southern Britain. This is not consistent with the scale type of southern Britain being monomorphic, and Scottish specimens having four or six distinct types (Warren 1961, 1968; Thomson 1970, 1980). Bowden (1983) described different scale types from different regions of the British Isles, raising doubts about the validity of any distinction between populations from Scotland and elsewhere. In particular the named subspecies *britannica* was described as having *thomsoni* type androconial scales. Bowden (1983) never conducted a statistical analysis of his categorical data. Such an analysis reveals that there is no significant difference between the frequencies of scale types in different regions ( $G = 10.50$ ;  $df = 15$ ;  $P > 0.05$ ) although such a comparison is not valid because according to our data distinct scale types do not exist. Even if distinct types cannot be identified using quantitative measures, the possibility of differences between the distributions of androconial shape

descriptors could exist if there was a distinction between regional types. Our analysis is not consistent with this hypothesis and there is no regional separation on the basis of androconial variation.

Quantification of wing morphological characteristics reveals a pattern that is far more complex than previously described. Separation of regional forms in a quantitative analysis is not consistent between generations. When quantified, variation of the characteristics that have been previously used to describe subspecies, including the separation of *sabellicae* in southern England from *napi* in mainland Europe, is not consistent with previous work. Those individual wing characteristics that have been used to describe subspecies do not vary between seasons or between regions in a consistent fashion. For example ventral ground colour, basal melanisation and vein colouration have been used to differentiate the nominal subspecies *sabellicae* from *napi*, and *thomsoni* and *britannica* from *sabellicae* (Stephens 1827; Verity 1916; Müller & Kautz 1939; Emmet 1989). Patterns of variation in these characteristics are not consistent between generations and when quantified, differences in one (basal melanisation) are the reverse of that which has previously been described. Whilst multivariate analysis shows some pattern in the differentiation of seasonal and regional forms of both sexes, there is also overlap in the morphology of regional forms. If regional separation of forms is possible, it has to be restricted to overall tendencies in individual characteristics but these characteristics are not necessarily correlated in how they vary, either with season or region.

The wing morphology of *Pieris napi* is recognised as being variable within regions and influenced by environmental conditions experienced in the pupal stage (Thompson 1947). The results presented here indicate that wing morphology characteristics, or at least those which have been used previously, lack the stability that would be required to use them to differentiate regional forms. Perhaps the most revealing result to emerge from this analysis is that some characteristics, especially size, yellow underside ground colouration and basal melanism are variable, and the most variable in northern populations. In *P. napi* these characteristics are of potential importance to thermoregulation, crypsis and flight performance (Wilcockson 2002). For example, reduced basal melanisation of southern French *P. napi* in comparison to the British Isles is consistent with an emphasis on thermal constraints on activity whilst small size in first generation and northern areas may facilitate rapid warming and maximise activity in cool conditions. A large size in southern France may also be consistent with reduced thermal constraints in warmer areas. Variability may be the result of a lack of directional selection in environments that vary in weather over short time scales. Thus, the greater variation within north-western populations may be explained by consistent within-season weather variation, which is more extreme than elsewhere. Whilst wing morphology variation does not resolve issues about levels of regional differentiation, controlled studies of reaction norms would reveal much about responses to selection on individual wing elements in variable environments. A more comprehensive analysis using more wing characteristics reveals the same pattern of variability as demonstrated here (Wilcockson 2002).

On the basis of androconial and wing morphology variation there seems to be little evidence for making any distinction between regional forms of *Pieris napi* in the Brit-

ish Isles. Furthermore there seems to be little basis for making any distinction between forms on the European mainland and the British Isles. Porter & Geiger (1995) examined  $F_{st}$  values derived from nine loci using populations throughout Europe, including Scotland and northern England. Their analysis revealed non-equilibrium amongst populations in the British Isles, which they attributed to either mixing of different forms or differences in local selection. Their analysis was designed to examine inferences of gene flow at different geographic scales, not specifically phylogenetic differentiation. But allozyme data has shown that many of the taxa in the *Pieris napi* complex lack a genetic justification. The use of such enzymes as markers for revealing phylogeography in a species in which there is likely to be considerable mobility (Porter & Geiger 1995; Asher *et al.* 2001), and which is possibly subject to different regional and local selection gradients, is unlikely to be conclusive. Whilst we are aware of arguments about the neutrality of allozymes we emphasise that at least one (PGI) is subject to selection in relation to thermal requirements in one other pierid butterfly (Kingsolver & Watt, 1984), and other commonly used allozymes are also involved in metabolic processes that could be under selection.

We conclude that on the basis of androconial and wing morphology characteristics there is no justification for raising any geographic forms within the British Isles to subspecific status. Direct evidence for any specific invasion sequence is also lacking and is unlikely to be obtained from allozyme data. Studies using the mitochondrial genome based on appropriate markers (microsatellites, RFLPs) are needed. Our studies of wing morphology also reveal that there is regional overlap, but within regions variation is greater in northern areas than elsewhere.

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